BEST AVAILABLE COPY

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C12Q 1/68, C12N 15/11, C07H 21/00 — A1

(11) International Publication Number: WO 93/24653

(43) International Publication Date: 9 December 1993 (09.12.93)

(21) International Application Number: PCT/EP93/01287

(22) International Filing Date: 21 May 1993 (21.05.93)

(30) Priority data:
MI92A001235 22 May 1992 (22.05.92) IT

(71) Applicant *(for all designated States except US)*: CONSIGLIO NAZIONALE DELLE RICERCHE [IT/IT]; Via Tiburtina, 770, I-00159 Rome (IT).

(72) Inventors; and

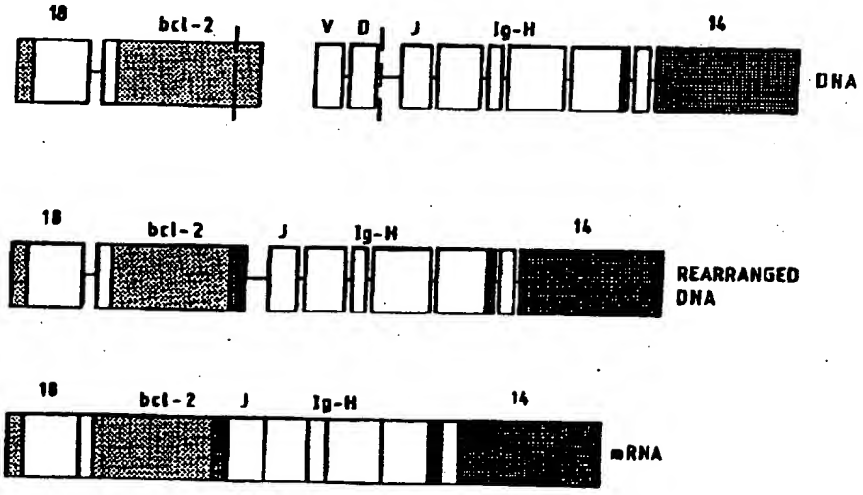
(75) Inventors/Applicants *(for US only)* : MORELLI, Susanna [IT/IT]; P.F. Applicazioni cliniche della ricerca oncologica del CNR, Via G. Venezian, 1, I-20133 Milan (IT). NICOLIN, Angelo [IT/IT]; Università degli Studi di Milano, Dipartimento di Farmacologia R10, Via Vanvitelli, 32, I-20129 Milan (IT).

(74) Agents: APPOLONI, Romano et al.; Ing. Barzanò & Zanardo Milano S.p.A., Via Borgonuovo, 10, I-20121 Milan (IT).

(81) Designated States: CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: ANTISENSE OLIGONUCLEOTIDES WITH ANTITUMORAL ACTIVITY, PHARMACEUTICAL COMPOSITIONS WHICH COMPRISE THEM, AND THEIR USES



(57) Abstract

The present invention relates to antisense oligonucleotides (aODN) which comprise nucleotidic sequences characterized by the possibility of hybridizing, also inside the interior of human cells, to regions of genetic code molecules originated from the translocation of a portion of chromosome 18 to other chromosomes, in particular chromosome 14. Chromosomal translocation is the cause of a large number of diseases, in particular tumors. The inhibition of the biological effects of translocations by antisense oligonucleotides is meaningful from the scientific knowledge, diagnostic and therapeutical viewpoints. The invention relates to the use of such antisense oligonucleotides in order to study the diagnostics and therapy of tumors originated from chromosomal translocation t(14; 18) and the chemical and pharmaceutical modification suitable for rendering them suitable for being administered to human subjects.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NL	Netherlands
BE	Belgium	GN	Guinea	NO	Norway
BF	Burkina Faso	GR	Greece	NZ	New Zealand
BG	Bulgaria	HU	Hungary	PL	Poland
BJ	Benin	IE	Ireland	PT	Portugal
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SK	Slovak Republic
CI	Côte d'Ivoire	KZ	Kazakhstan	SN	Senegal
CM	Cameroon	LI	Liechtenstein	SU	Soviet Union
CS	Czechoslovakia	LK	Sri Lanka	TD	Chad
CZ	Czech Republic	LU	Luxembourg	TG	Togo
DE	Germany	MC	Monaco	UA	Ukraine
DK	Denmark	MG	Madagascar	US	United States of America
ES	Spain	ML	Mali	VN	Viet Nam
FI	Finland	MN	Mongolia		

"ANTISENSE OLIGONUCLEOTIDES WITH ANTITUMORAL ACTIVITY, PHARMACEUTICAL COMPOSITIONS WHICH COMPRISE THEM, AND THEIR USES"

The present invention relates to antisense oligonucleotides, in particular antisense oligonucleotides which block the synthesis of BCL 2 protein.

The traditional obstacle in tumor chemotherapy resides in the difficulty of rigorously identifying cancer-specific targets. The therapeutical use of even active compounds displaying a very selective mechanism of action against specific molecular targets does not succeed in differentiating, to a desired extent, the effect towards neoplastic cells and healthy cells.

During the past years, alterations in genetic code appeared with increasing evidence to be responsible for neoplastic transformation and progression. The molecular alterations relate to punctiform mutations, amplifications or deletions, genic translocations between chromosomes. Such molecular anomalies determine the neoplastic outcome by means of one single mechanism, or, more often, through the successive contributions by a plurality of alterations.

In a large number of tumoral situations, in particular of hematologic origin the main tumorigenic alterations were identified. In such cases sequences of genetic code were identified, which may often be very short or constituted by one single replaced base, which not only result to be cancer-specific, but also,

frequently, tumor-specific.

The inhibition of the functions of the genes affected by the alterations, with the normal gene being kept untouched, would make it possible remedies to be found for the damages caused by the altered gene, with the normal gene being kept unaltered. On considering that the altered gene is exclusively expressed in tumoral cells, the effect of such an inhibition would only be exerted onto the neoplastic cell, with a cancer-specific therapy being actually realized.

The possibility of inhibiting the genic functions by means of a very specific interaction with a short portion of nucleotidic sequence of the same gene is offered by the antisense oligonucleotides. The antisense oligonucleotides are constituted by short chains of single-filament synthetic DNA. The order of sequence of the single bases is so designed as to result complementary with the sequence of messenger RNA which one wants to inhibit.

The strict dependence on a perfect complementarity with the target molecule determines the genic expression inhibiting effect displayed by the antisense compounds. Therefore, the antisense compounds offer themselves as potential antitumoral compounds in those cases in which the molecular alterations are known.

On considering the above, the subject-matter of the present invention are antisense oligonucleotides (aODN's) characterized in that they comprise

nucleotidic sequences hybridizing to a sequence of the hybrid gene and its messenger at the splicing region which is formed following a translocation of bcl-2 gene from chromosome 18 to chromosome 14 in a section of the latter which contains Ig-H gene (the gene for immunoglobulin H), and which block the synthesis of human BCL-2 protein.

Therefore, the present invention relates to the use of antisense oligonucleotides (aODN's) for the study and therapy of tumors in which BCL 2 protein is produced in excessive amounts owing to an alteration in the genetic code. In particular, the alteration consists in the translocation of a portion of chromosome 18 containing bcp-2 gene into a portion of chromosome 14 containing Ig-H gene. The splicing point between said portions forms a nucleotidic sequence only present in tumoral cells.

Such a mechanism is schematically displayed in Figure 1 of the accompanying drawings.

According to the present invention, antisense oligonucleotides complementary to the sequence formed owing to the translocation are capable of inhibiting the expression of BCL 2 protein in tumoral cells only, actually causing a growth inhibition and a cellular death of said cells with simultaneously respecting the integrity of the healthy cells.

A further subject-matter of the present invention is also a phramaceutical composition containing at least one antisense oligonucleotide as defined hereinabove, or a chemically modified form thereof

without that the nucleotidic sequence is altered, and a therapeutically acceptable carrier.

A last subject-matter of the present invention is the use of such antisense compounds or of modified forms thereof in order to prepare a pharmaceutical composition to be used for the treatment of some pathological forms, in particular some tumor types.

The aODN's of the present invention can be designed on the basis of the sequence of mRNA in the splicing region between bcl-2 gene and the gene for the heavy chain of immunoglobulin Ig-H. Said sequence may be either known or determined by sequentiating samples of tumoral lymphocytes according to standardized methodologies as disclosed in a large number of manuals (Molecular Cloning, A Laboratory Manual, 1989, Sambrook, Maniatis, etc.).

For the purposes of the present invention, only those aODN's are understood as being suitable, which have a specific length suitable for hybridizing in optimal way to mRNA and target DNA. Such a length may also vary as a function of the chemical modifications introduced into the molecule constituted by natural nucleotides in order to enhance the pharmacological properties thereof. The minimal length preferably is 10 nucleotides, and the maximal length is of 100 nucleotides, preferably 50, still more preferably 30 nucleotides. The above, also on considering several mechanisms which may determine the biological effect.

Antisense compounds may act in various moments of the metabolic history of mRNA, both at nuclear and

cytoplasmatic levels. It is furthermore possible that one from the antisense compounds action points is at ribosome level, or they may directly act through a direct interaction with nuclear or mitochondrial DNA.

The sequence length can also be determined on the basis of considerations known to those skilled in the art, which relate to a sufficient effectiveness of penetration through the cellular membranes. (Locke et al. PNAS 86:3474, 1989, Yakubov et al. PNAS 86: 6454, 1989).

A specific sequence defined according to such criteria as cited hereinabove can be synthetized in solid phase by means of well-known methods in the art and described, for example, by Narang (Tetrahedron 39:3, 1983), or by Itakuran (Ann. Rev. Biochem. 53:323, 1984), or in "Oligonucleotide Synthesis; A Practical Approach", Gait M. J. Ed. IRL Press, Oxford, U.K., 1984).

If so desired, such aODN's can be purified by means of known methods in the art, such as, e.g., polyacrylamide gel electrophoresis under denaturing conditions, or by high resolution chromatography, reverse phase chromatography or ion exchange column chromatography, or, finally, by capillary electrophoresis.

The aODN's according to the present invention are characterized by the possibility of inhibiting the synthesis of BCL 2 protein in the tumoral cells which display the t(14;18) translocation, by means of the use of specific antibodies, or by taking advantage

from the capability of inhibiting the relevant mRNA by means of molecular probes. Also the inhibition of proteinic translation can be caused by means of a translation in cell-free system using lysates of red cells or wheat germ extracts (Sambrook, etc.).

The inhibition of synthesis of BCL 2 protein can also be determined according to a dose-effect relationship, by evaluating the growth inhibition of human tumor cells bearing the t(14;18) translocation, by evaluating the number of cells under the optical microscope or by means of the tritiated thymidine incorporation test.

The activity of the compounds according to the present invention can also be determined by analysing the induction of orderly cellular deletion in the already cited tumoral cells. The orderly cellular deletion, or apoptosis, is determined by analysing the cellular nucleus, or carrying out an electrophoresis in agarose, of the nuclear extract which appears to be fragmented into units of approximately 200 bases.

The aODN's according to the present invention can be modified for the purpose of causing the desired characteristics thereof to be improved (for a general survey also as regards general considerations relating to designing antisense oligonucleotides for the purpose of blocking the synthesis of a specific protein, reference is made to Uhlmann E. and Peymann A. Chem. Rev. 90:5543, 1990 or Englisch U. and Gauss D.H. Angewandte Chemie 30:613, 1991).

For example, such aODN's can be chemically

modified at the phosphodiester linkage, or at the terminal 3' ends in order to render them more stable against a cleavage by nuclease enzymes, or in order to improve their penetration or hybridization characteristics, as disclosed in European Patent Application published with Publication No. 386 563. Furthermore, the aODN's can be covalently bound to a lipid, as disclosed, e.g., in International Patent Application published with Publication No. WO/9010448. In such a way, the concerned aODN can be transported more effectively through the cellular membranes and can be cleft, for example, with cellular cytoplasmatic enzymes in order to liberate active aODN.

In order to obtain a higher absorption rate of aODN's into the cells or in order to improve the oral absorption thereof, the aODN's may also be phosphorylated, or they may be linked to cholesterin or to cholesterol derivatives, at their ends (Letsinger et. al. PNAS 86:6553, 1989).

When the aODN's are modified in order to increase their stability against an enzymatic cleavage or in order to improve the absorption thereof, care should be taken in order to make sure that such modified aODN's will still hybridize well to the specific mRNA or DNA, in order that they retain their biological and pharmacological effect. Furthermore, the aODN's of the present invention may be modified in order to secure that the drug is specifically delivered to tumoral tissues and consequently the dispersion of administered molecule is limited.

According to a further aspect of the present invention, the aODN's according to the present invention may be administered in acceptable pharmaceutical forms, in particular in a suitable form for an oral administration. Dosage forms and rhythms can be parallel to those as adopted in clinical administrations of known compounds. The pharmaceutical compositions may contain at least one aODN according to the present invention in association with active substances and with either solid or liquid, pharmaceutically acceptable carriers. Any suitable carriers may be used. Furthermore, the pharmaceutical compositions may contain further pharmaceutically active substances and can be prepared according to known methods in the art.

Furthermore, the use of aODN's according to the present invention or modified forms thereof in order to prepare such pharmaceutical compositions and for diagnostic or medical purposes, for example for the treatment of some tumoral forms or of other diseases which may benefit from an inhibition of the synthesis of BCL 2 protein, are also a subject-matter of the present invention.

Furthermore, the aODN's of the present invention can be used, after being fixed onto a solid carrier according to well known methods in ther art, in order to detect genetic alterations responsible for various pathological forms, including tumors. For such diagnostic purposes, the aODN's according to the present invention may be used with a signal portion,

for example a radioactive isotope, an enzyme, or a fluorescer according to well known methods in the art.

The present invention having thus being generally disclosed, it will be better understood by referring to the following specific examples, which are reported hereinunder for merely illustrative purposes, and which in no way shall be construed as being limitative of the same invention.

Examples

Example 1

Synthesis of an aODN

An aODN according to the present invention, with the following sequence, was synthetized by using a Beckman DNA synthetizer:

5' GGT-CCG-AGC-TTG-ACT-ACT 3'

Such a sequence is displayed in greater detail in Figure 2 of the accompanying drawings.

Example 2

Activity of an aODN

On day 1, human DHL-4 cells were grown in HB 101 media with a density of 10^4 cells per each well, to which an aODN according to the present invention was added in an amount comprised within the range of from 1 to 10 micromoles (microM).

On the following days, the cultures were admixed with half aODN dose and the activity was tested on from 2 to 7 days by cell count by microscope examination followed by a count of radioactivity incorporated into DNA following the addition of tritiated thymidine. The aODN's demonstrated a clear

capability of reducing both the number of cells in the wells and the amount of radioactivity incorporated to cell nuclei. The activity results to be dose-dependent and specific to cells bearing the genetic damage to which aODN was directed.

In this regard, reference is made to Figures from 3 to 5 of the accompanying drawings.

Figure 3 shows the effect of an aODN on the in vitro growth of a human tumor cell line DHL-4. The growth curves respectively relate to the untreated DHL-4 line, to the same line treated with an aODN constituted by a sense sequence, a casual sequence, or the same line DHL-4 treated with an antisense sequence. The reported data relates to the dose of 1 microM.

On the ordinate, the number of cells x 10^3 is reported, which represents the total cells count. On the abscissa, the time is reported in days. The four curves relate to: I: reversed control, i.e., a polynucleotide having a reversed nucleotidic sequence relatively to the antisense nucleotide used in the test; U: untreated cells; S: sense nucleotide (control); AS: antisense nucleotide according to the present invention.

From the trend of the lines shown in Figure 3, it can be understood that the inhibition effect is practically inexistent for the control and untreated curves, whilst is of considerable extent for the antisense oligonucleotide according to the present invention.

Figure 4 displays the dose-dependent effect of aODN on the growth of DHL-4 tumor. The doses are indicated as concentrations (from 10 microM down to 0.001 microM) of aODN used. From the chart of Figure 4, one may understand how a decrease in the activity of inhibition of DHL-4 tumor cell growth is displayed with decreasing antisense oligonucleotide dose administered.

Figure 5 shows the specific antiproliferative effect for tumor cells bearing the genetic damage against which aODN is directed. No growth inhibiting effects are exerted against human tumor cells not bearing the molecular alteration which is the target of the present aODN.

In the chart of Figure 5, displayed on the ordinate is the number of cells x 10^3 which represents the total cell count. On the abscissa, the cases for as many tumor types are reported: DHL-4 is the tumor of leukemia B with 14-18 translocation, which is relevant for the purposes of the present invention; Raji is a leukemia B tumor without 14-18 translocation. LCL represents the tumor of leukemia B without translocation, YAC is the leukemia T tumor without translocation, and, finally, K562 represents the tumor of mieloid leukemia without translocation.

For each tumor type, reported are four different cases of administration of doses of 10 microM and 1 microM for antisense (AS) and sense (S) oligonucleotides, as explained above.

From such a chart, one may see how an absolute

selectivity is obtained in the effect of growth inhibition on tumor DHL-4 only, so that the use of antisense oligonucleotides according to the present invention makes it possible only such a kind of growth to be inhibited, without influencing, on the contrary, the growth of the other tumor types, which depart from the scope of the present invention.

Example 3

Induction of apoptosis

The above said cells, after being treated with the aODN's according to the present invention, result (a) to modify their morphological appearance, (b) to demonstrate DNA fragmentation and (c) to display an at all abnormal distribution of their DNA content.

Figure 6 of the accompanying drawings represents, in that regard, a cytofluorimetric analysis of cellular DNA, relevant to the effect of DNA fragmentation caused by the compounds. In this regard, the chart "A" relates to the control, in the case of untreated cells. The chart "B" relates to cells treated with an antisense oligonucleotide according to the present invention. The chart "C" relates to the overlapping of the histograms relevant to the cases of untreated cells and of cells treated with the antisense oligonucleotide. Finally, the chart "D" is obtained by overlapping the histogram "B" to the histogram of cells treated with sense oligonucleotide.

From the trend of such charts, it is evident that the effect of DNA fragmentation is only obtained with the case of treatment by means of antisense

oligonucleotide according to the invention. As known, an effect of DNA fragmentation means that a mechanism of apoptosis has occurred. We wish to remind that in this kind of charts on the ordinate the number of cells is reported, and on the abscissa the intensity of fluorescence is expressed.

After classic staining with May Grundwall Giemsa, the cells display a fragmented nuclear appearance, and at all recognizable when untreated cells or cells treated with an ODN without antisense activity, or without an activity correlated with the genetic alteration taken into consideration for the purposes of the present invention, are tested for nuclear morphology. The tumor treated as already mentioned is submitted to nuclear DNA extraction according to well known methods in the art and is submitted to electrophoresis in agarose by following a standardized methodology (also described by Maniatis).

On electrophoretic examination, the DNA of the tumor treated with an aODN according to the present invention appears to have been fragmented into bands which are regularly ditributed in agarose gel and correspond to 200-base segments and are detected by U.V. light examination.

Also on flux cytofluorimetric examination, the already mentioned cells, after treatment with an aODN according to the present invention appear to be of reduced volume as compared to control cells and, after their DNA being stained with ethidium bromide, when submitted to the same cytofluorimetric examination

appear to contain a reduced DNA amount, so that most cells result to emit a lower fluorescence than as corresponding to normal DNA content.

All these evidences demonstrate that the aODN's according to the present invention are capable of inducing an orderly cell deletion (apoptosis), and that said orderly cell deletion results to be important for the pharmacological activity disclosed hereinabove.

C l a i m s

1. Antisense oligonucleotides (aODN's) characterized in that they comprise nucleotidic sequences hybridizing to a sequence of the hybrid gene and its messenger at the splicing zone which is formed following a translocation of bcl-2 gene from chromosome 18 to chromosome 14 in a section of the latter which contains Ig-H gene (the gene for immunoglobulin H), and which block the synthesis of human BCL-2 protein.

2. Antisense oligonucleotides according to claim 1, characterized in that they comprise a nucleotidic sequence including at least one portion of bcl-2 gene and at least one portion of the gene of immunoglobulin H.

3. Antisense oligonucleotides according to claim 1, characterized in that they have a length of not more than 100 nucleotides.

4. Antisense oligonucleotides according to claim 2, characterized in that they have a length comprised within the range of from 10 to 50 nucleotides.

5. Oligonucleotides according to claim 1, characterized in that they comprise the following nucleotidic sequence:

5' GGT-CCG-AGC-TTG-ACT-ACT 3'

6. Pharmaceutical composition containing at least one antisense oligonucleotide according to claim 1 and a therapeutically acceptable carrier substance.

7. Use of an antisense oligonucleotide according to claim 1 for preparing a pharmaceutical composition.

8. Use of a sequence totally or partially corresponding to the hybrid gene resulting from the translocation of human bcl-2 gene to chromosome 14, inside the immunoglobulin gene, to be stably inserted in "antisense" direction inside the genoma of human cells, for therapeutical purpose.

9. Use of an antisense oligonucleotide probe, as claimed according to any of the preceding claims, as a diagnostic and therapeutical agent.

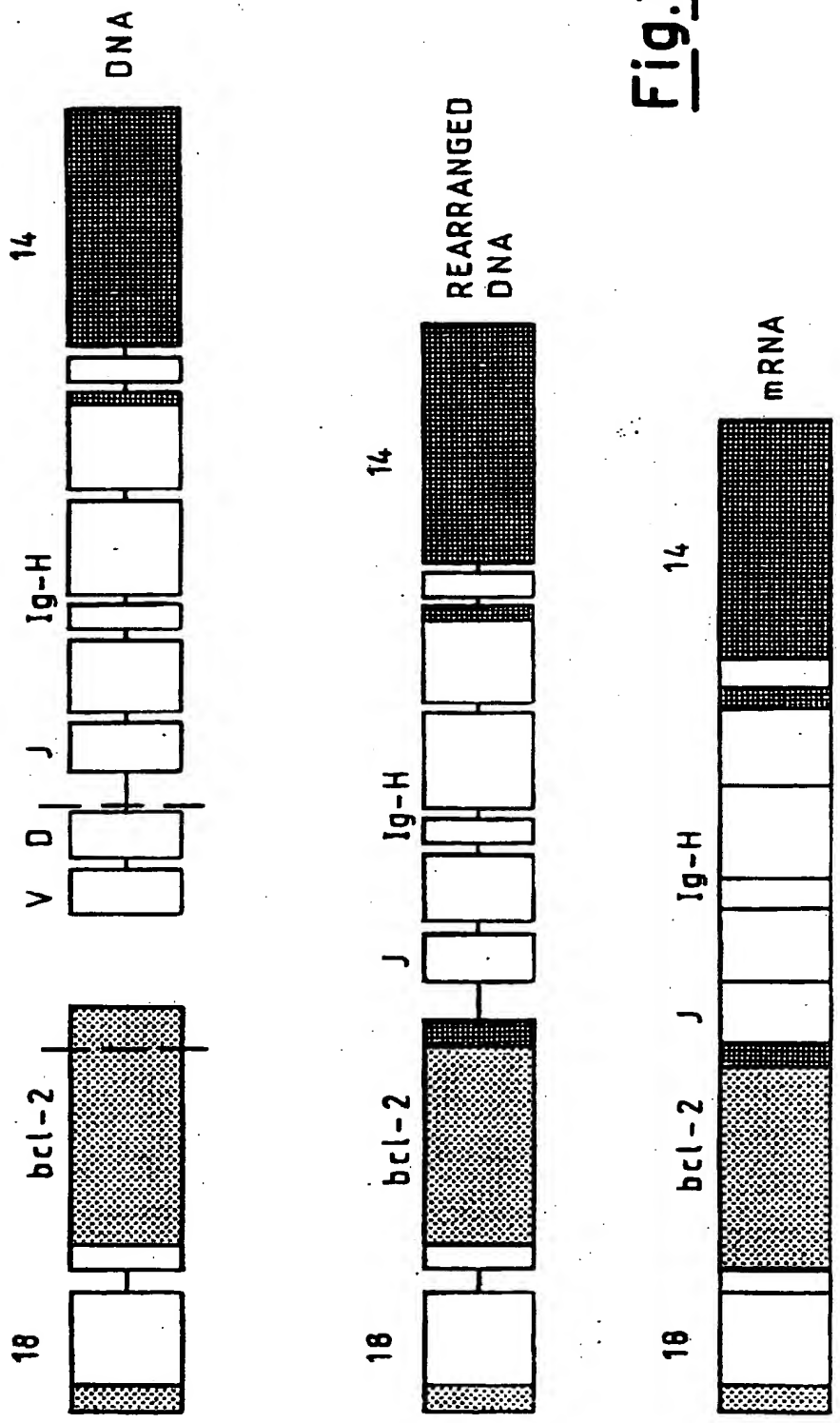

DNA
14
Ig-H
J
D
V
bcl-2
18
REARRANGED DNA
14
Ig-H
J
bcl-2
18
mRNA
14
Ig-H
J
bcl-2
18

Fig.1

Fig.2

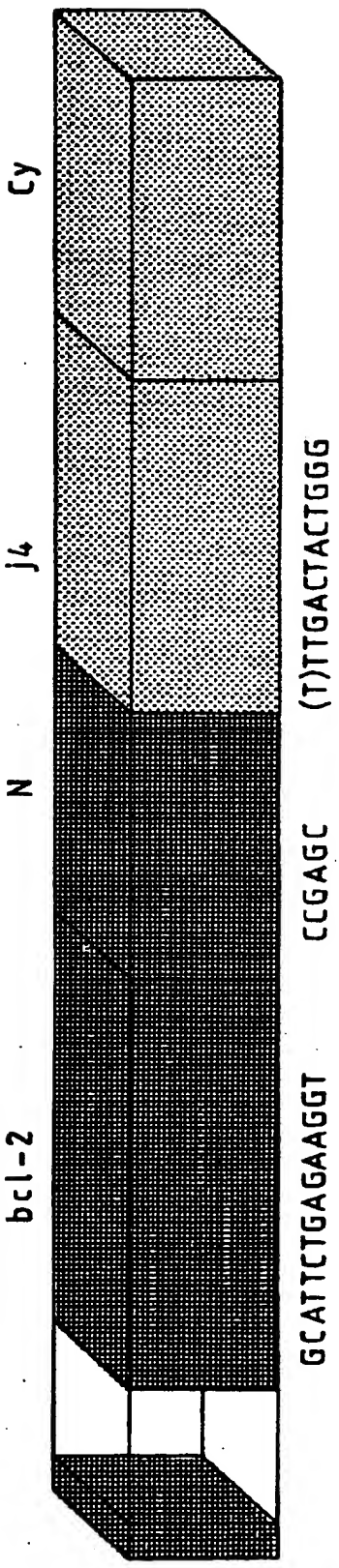


Fig.3

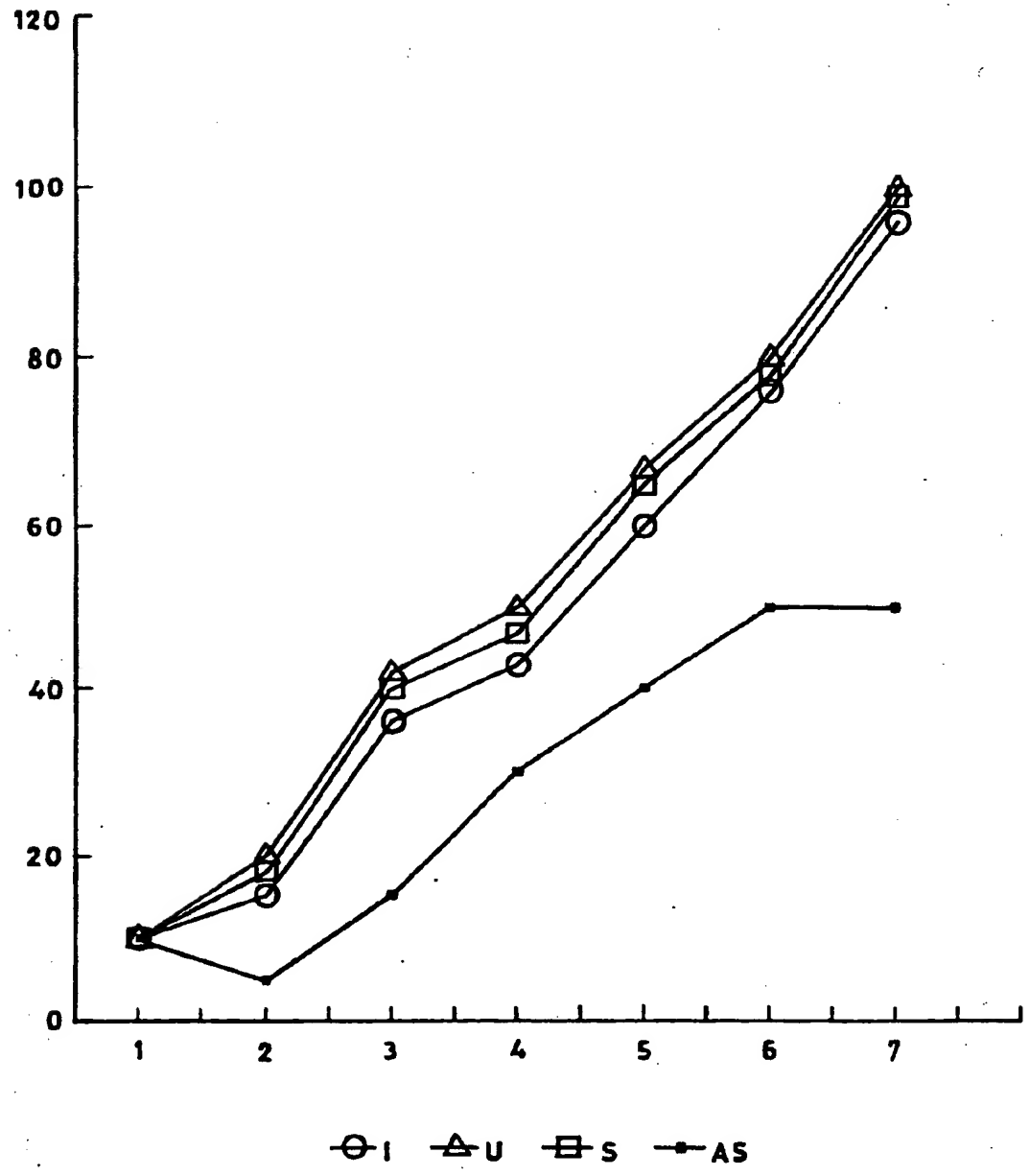


Fig.4

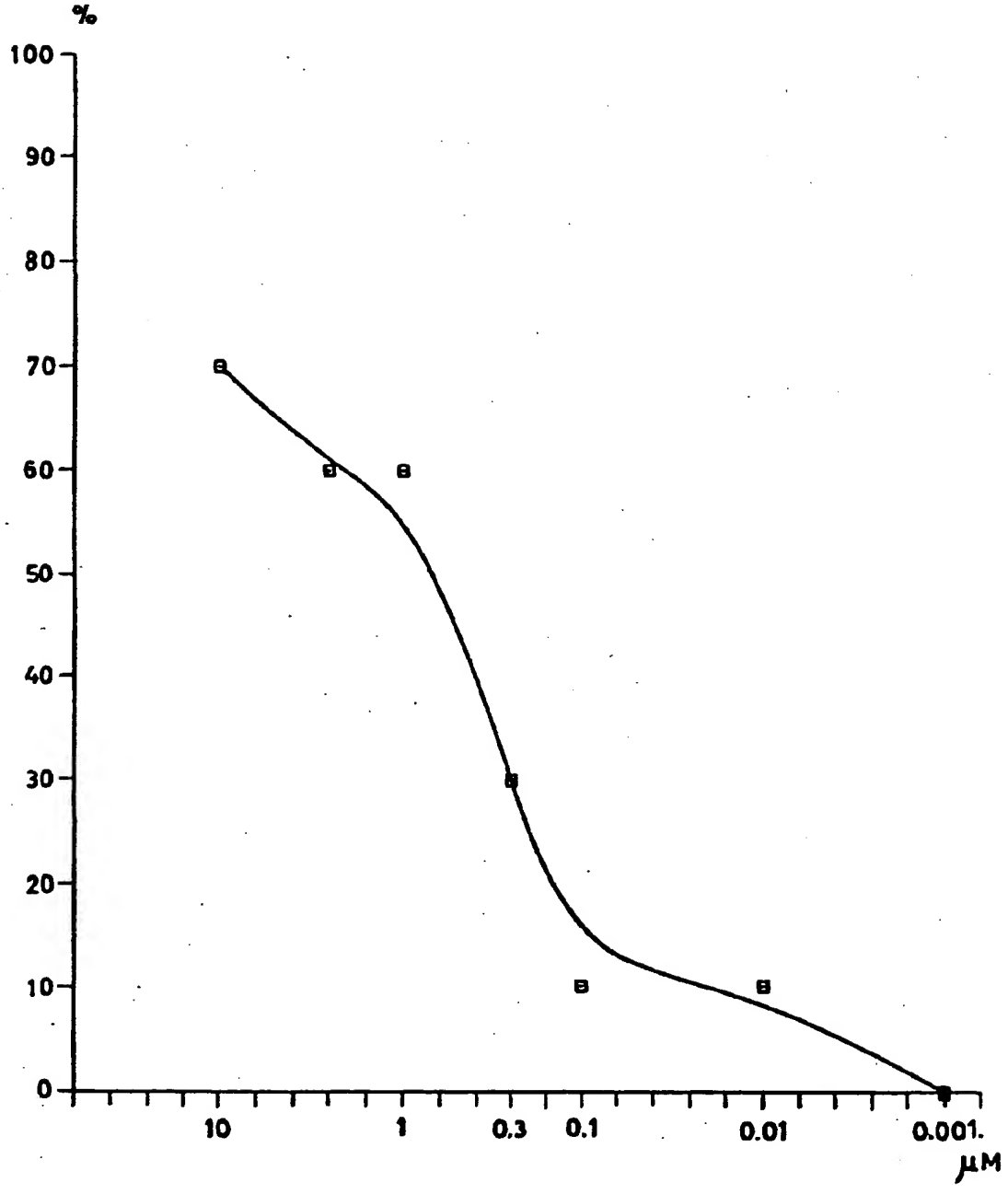


Fig.5

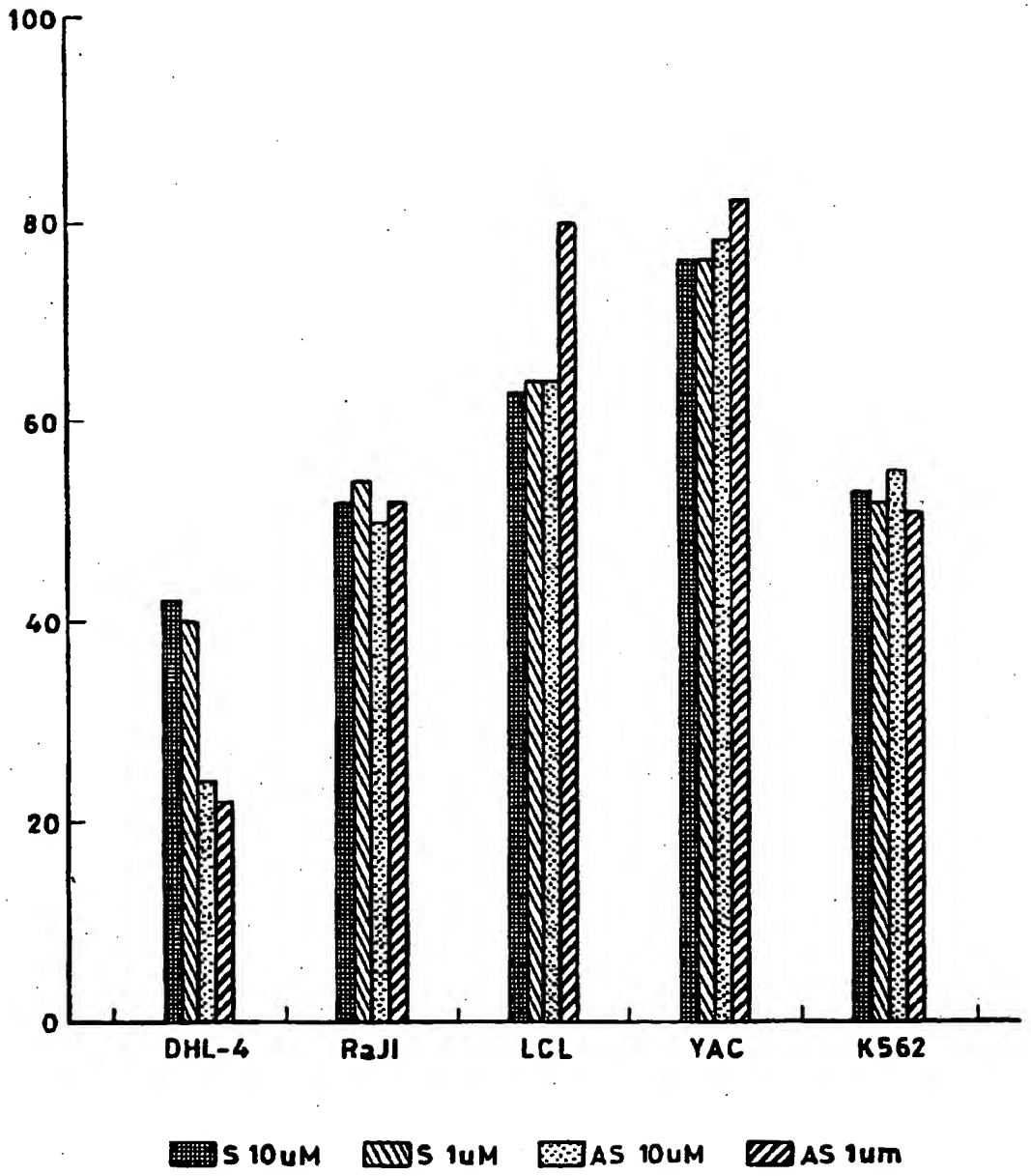
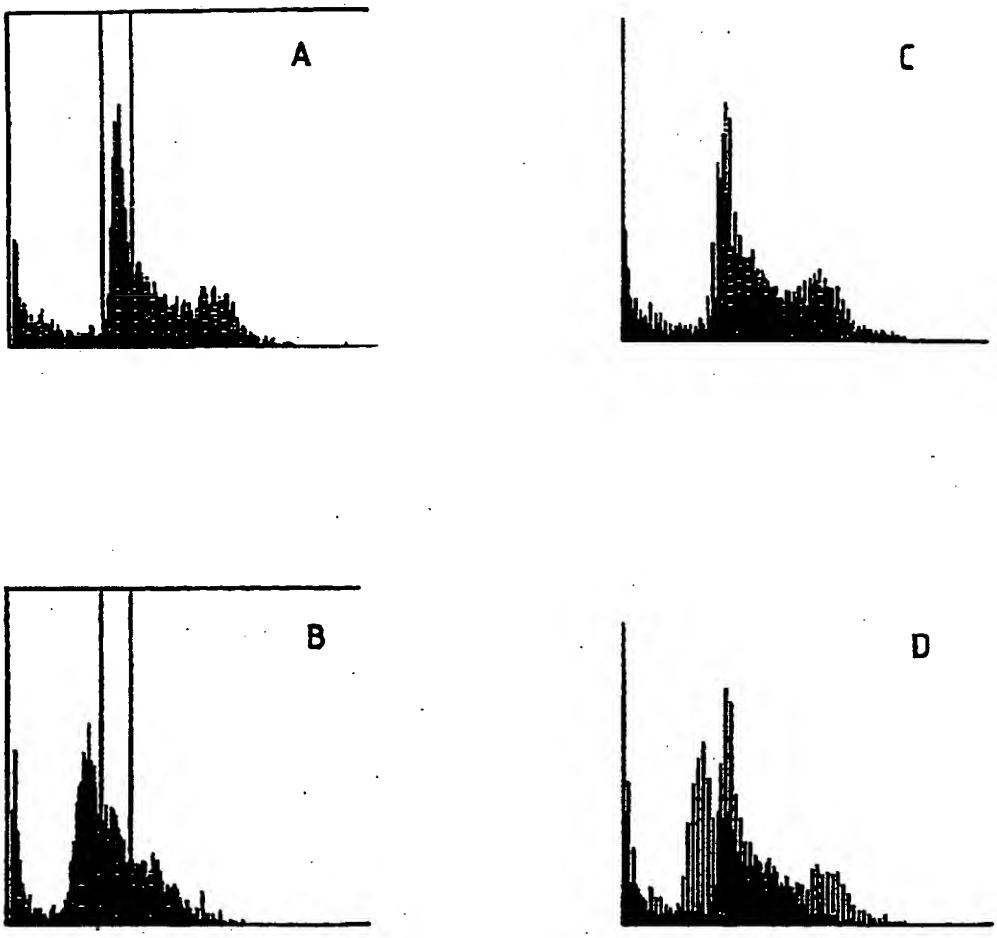

100
80
60
40
20
0
DHL-4
RaJI
LCL
YAC
K562
S 10uM
S 1uM
AS 10uM
AS 1um

Fig.6



INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 93/01287

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12Q1/68; C12N15/11; C07H21/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12Q ; C12N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[10]	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	WO,A,9 205 272 (THE BOARD OF REGENTS OF THE UNIVERSITY OF CALIFORNIA) 2 April 1992 see the whole document ---	1-9
A	BIOLOGICAL ABSTRACTS vol. 91, no. 4 , 1991, Philadelphia, PA, US; abstract no. 40883, ADACHI ET AL. 'Potential Z-DNA elements surround the breakpoints of chromosome translocation within the flanking region of bcl-2 gene' see abstract & ONCOGENE 5 (11). 1990. 1653-1658. --- -/--	1-9

* Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
29 SEPTEMBER 1993	13. 10. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	MOLINA GALAN E.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	BIOLOGICAL ABSTRACTS vol. 92, no. 11 , 1991, Philadelphia, PA, US; abstract no. 126202, ZELENETZ A D; CHU G; GALILI N; BANGS C D; HORNING S J; DONLON T A; CLEARY M L; LEVY R 'ENHANCED DETECTION OF THE T 14 18 TRANSLOCATION IN MALIGNANT LYMPHOMA USING PULSED-FIELD GEL ELECTROPHORESIS.' see abstract & BLOOD 78 (6). 1991. 1552-1560. ---	1-9
A	EP,A,0 252 685 (WISTAR CORP.) 13 January 1988 ---	-
A	SCIENCE vol. 228, 21 June 1985, LANCASTER, PA US pages 1440 - 1443 TSUJIMOTO ET AL. 'Involvement of the bcl-2 gene in human follicular lymphoma' ---	-
P,X	WO,A,9 222 303 (TEMPLE UNIV.) 23 December 1992 see the whole document -----	1-4,6-9

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

EP 9301287
SA 74548

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 29/09/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9205272	02-04-92	CA-A- 2091759	18-03-92
		EP-A- 0556231	25-08-93
EP-A-0252685	13-01-88	US-A- 5015568	14-05-91
		AU-B- 602704	25-10-90
		AU-A- 7532887	18-02-88
		DE-A- 3786200	22-07-93
		JP-A- 63100379	02-05-88
		US-A- 5202429	13-04-93
WO-A-9222303	23-12-92	None	

EPO FORM P079

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☑ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.